

Listing of Claims

This listing of claims will replace all prior versions and listings of claims in this application.

1. [Currently Amended] Method for the preparation of a strain of evolved micro-organisms for the production of 1,2-propanediol by the metabolism of a simple carbon source, said which method comprising growing comprises the growth an initial bacterial strain, under selection pressure in an appropriate growth medium comprising containing a simple carbon source, said initial bacterial strain that has undergone comprising a deletion of the gene *tpiA* and the a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, in order to cause evolution to evolve, in said initial strain, of one or more genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol towards evolved genes having that possess an improved "1,2-propanediol synthase" activity, which resulting then selecting and isolating evolved strain or strains of evolved micro-organisms that possess having an improved "1,2-propanediol synthase" activity are then selected and isolated.
2. [Currently Amended] ~~Method according to Claim 1, characterised in that~~ The method of claim 1, wherein the gene involved in the conversion of methylglyoxal into lactate is selected from the group consisting in *gloA*, *aldA* and *or aldB*.
3. [Currently Amended] ~~Method according to either of Claims 1 or 2, characterized in that~~ The method of claim 1, wherein the initial strain ~~has undergone the~~ comprises deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
4. [Currently Amended] ~~Method according to any of Claims 1 to 3, characterized in that~~ The method of claim 1, wherein the initial strain ~~has also undergone the~~ comprises deletion of the genes *IdhA*, *pflA*, *pflB*, *adhE* and *edd*.

5. [Currently Amended] ~~Method according to any of Claims to 4, characterized in that~~ The method of claim 1, wherein the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
6. [Currently Amended] ~~Method according to Claim 5, characterized in that~~ The method of claim 1, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
7. [Currently Amended] ~~Method according to either of Claims 5 or 6, characterized in that~~ The method of claim 5, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.
8. [Currently Amended] ~~Method according to Claim 7, characterized in that~~ The method of claim 7, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
9. [Currently Amended] ~~Method according to any of Claims 6 to 8, characterised in that~~ The method of claim 6, wherein the enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme.
10. [Currently Amended] ~~Method according to any of Claims 1 to 9, characterised in that~~ The method of claim 1, wherein one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone are introduced into the evolved ~~strain~~ microorganisms.
11. [Currently Amended] ~~Method according to Claim 10, characterised in that~~ The method of claim 10, wherein one the heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate are from *C. acetobutylicum*.

12. [Currently Amended] ~~Method according to either of Claims 10 or 11,~~
~~characterised in that~~ The method of claim 10, wherein an evolved the modified
evolved strain comprising one or more heterologous genes coding for one or more
enzymes involved in the conversion of acetyl-CoA and acetate into acetone
~~obtained according to either of Claims 10 or 11~~ is grown under selection pressure
in an appropriate growth medium ~~containing~~ comprising a simple carbon source
in order to cause, in said evolved modified evolved strain, the evolution of one or
more genes involved in the conversion of acetyl-CoA and acetate to acetone
towards an improved "acetone synthase" activity ~~The, the~~ second generation of
resulting evolved micro-organisms ~~that possess~~ having an improved "1,2-
propanediol synthase" activity and an improved "acetone synthase" activity are
then selected and isolated.
13. [Currently Amended] ~~Method according to any of the preceding claims,~~
~~characterised in that~~ The method of claim 1, wherein the strain is selected from
the group consisting of a strain of bacterium, a yeast and ~~or~~ a fungus.
14. [Currently Amended] ~~Method according to Claim 13, characterised in that~~
The method of claim 13, wherein the strain is selected from the group consisting
of a strain of Escherichia, in particular E.coli, and Corynebacterium, in
particular C. glutamicum.
15. [Cancelled].
16. [Currently amended] Evolved strain that can be obtained by the method according
to any of Claims 1 to 14.
17. [Original] Strain according to Claim 16, in which the gene *Ipd* has a point mutation
whereby alanine 55 is replaced by valine.

18. [Currently amended] Method of preparation of 1,2-propanediol ~~in which~~ wherein an evolved strain of claim 16 is grown ~~according to either of Claims 16 or 17~~ in an appropriate growth medium containing a simple carbon source, and wherein ~~in which~~ the 1,2-propanediol produced is recovered.
19. [Currently amended] ~~Method according to Claim 18, characterised in that~~ The method of claim 18, wherein 1,2-propanediol and acetone are recovered.
20. [Currently amended] ~~Method according to either of Claims 18 or 19, characterised in that~~ The method of claim 18, wherein 1,2-propanediol and/or acetone are purified.
21. [New] The method of claim 14, wherein the strain is selected among the group consisting of *E. coli*, and *C. glutamicum*.
22. [New] Initial bacterial strain of a microorganism comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate.
23. [New] The strain of claim 22, wherein the gene involved in the conversion of methylglyoxal into lactate is selected among the group consisting in *gloA*, *aldA* and *aldB*.
24. [New] The method of claim 22, wherein the initial strain comprises deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
25. [New] The strain of claim 22, wherein the initial strain comprises deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.
26. [New] The strain of claim 22, wherein the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
27. [New] The strain of claim 22, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.

28. [New] The strain of claim 27, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.
29. [New] The strain of claim 27, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
30. [New] The strain of claim 22, selected from the group consisting of a bacterium, a yeast and a fungus.
31. [New] The strain of claim 30, selected from the group consisting of *Escherichia* and *Corynebacterium*.
32. [New] The strain of claim 16, comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, selected from the group consisting in *gloA*, *aldA* and *aldB*.
33. [New] The strain of claim 16, comprising deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
34. [New] The strain of claim 16, comprising deletion of the genes *IdhA*, *pflA*, *pflB*, *adhE* and *edd*.
35. [New] The strain of claim 16, comprising at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
36. [New] The strain of claim 36, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
37. [New] The strain of claim 36, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.

38. [New] The strain of claim 37, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
39. [[New] The strain of claim 36, wherein the enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme.
40. [New] The strain of claim 16, comprising one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone.
41. [New] The strain of claim 40, wherein one the heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate is from *C. acetobutylicum*.
42. [New] The strain of claim 16, selected from the group consisting of a bacterium, a yeast and a fungus.
43. [New] The strain of claim 16, selected from the group consisting of *Escherichia*, and *Corynebacterium*.
44. [New] The strain of claim 17, selected from the group consisting of a bacterium, a yeast and a fungus.
45. [New] The strain of claim 17, selected from the group consisting of *Escherichia*, and *Corynebacterium*.
46. [New] Evolved strain that can be obtained by the method of Claim 10.
47. [New] The strain of Claim 46, in which the gene *Ipd* has a point mutation whereby alanine 55 is replaced by valine.
48. [New] The strain of claim 46, selected from the group consisting of a bacterium, a yeast and a fungus.
49. New] The strain of claim 46, selected from the group consisting of *Escherichia* and *Corynebacterium*.